- **WORK PLAN & METHODOLOGY**

Methodology:

Most of the crude drugs (Plant materials) are usually put in quarantine store and they remain there for long time. During storage proper ventilation, humidity controls, suitable temperature and light conditions should be ensured to maintain their original pharmacological action. However, it is observed that, crude plant materials, before being taken for processing, are not analyzed which can lead to changes in original characteristics. To avoid this, the crude drugs should be tested for the following tests as per the USP and Indian Herbal Pharmacopeia (IHP).

1. Foreign organic matter.
2. Ethanol soluble extractives.
3. Water soluble extractives.
4. Total ash contents.
5. Acid insoluble ash.
6. Water soluble ash.
7. Loss on drying
8. Percentage moisture content.

Proximate analysis of plant material of *Abutilon indicum* is carries optimization of extraction condition of plant material will study for different solvents. Plant constituents of medicinal importance from an extensively diverse group of chemical compounds showing greater variation in solubility and stability. These are fixed Oils, fats and waxes, phenols, Tannins, Proteins, alkaloids, Carbohydrates, glycosides, Volatile oils, Resins and Resin Combinations.

In present study the phytochemical analysis of *Abutilon indicum* will be carried out by extraction method. The classical chemical procedure for obtaining constituents from dried plant tissues is to continuously extract powdered material in soxhlet apparatus. The observation of phytochemical extracts like moderately polar extract of terpenoids and phenolic, basic extract of alkaloids, polar extract of quaternary alkaloids and n-oxides, neutral extract of fats and waxes and fibers used for calculation of percent extraction. After complete phytochemical profile of given plant material fractionation of crude extract is desirable in order to separate the main classes of constituents from each other prior to chromatographic analysis.

The phytochemicals can be broadly classified as fixed oils, fats and waxes (lipids), phenols, Tanis, proteins, alkaloids carbohydrates glycosides volatiles oils, resin and combinations. The precise modern of extraction depends on the tenure and type of the substance isolated. The classical chemical procedure for obtaining constituents from dried plants tissues is to continuously extract powdered material by Soxhlet apparatus with a range of solvents.

The plant material will be cleaned, washed and dried in shades. After complete drying, the dry roots are kept in oven at 30^0C for one week and then powdered (sieved by 80 mesh size.) and use for further analysis.

Following steps will be used for phytochemical analysis:

1) Accurately weighed 5 gm of the whole plant powder will be packed in Whatman filter paper and kept in soxhlet apparatus for continuous extraction for 12 hr. The mixture of methanol and water 125 ml in volume ratio 4:1 used as extract. The extract will be cooled and filtration through whatman filter paper no. 41 into a dry, pre-weighed beaker.
2) The residue with 125CC of (5 x 25 CC) of ethyl acetate and filtered into a dry pre-weighed beaker. The residue obtained after filtration comprised plant fibers.
3) The filtrate obtain evaporate to dryness on a water bath maintained at 45 ^{0}C $\pm$ 5 ^{o}C after evaporation of EtOAc the beaker is allowed to cool at room temp in dessicator. After cooling the weight of beaker containing the residue is note down. The residue in EtOAc to obtain a final concentration of 10mg/cc. this extract is filtered through W. filter paper No. 41. The filtrate is neutral extract. It consists of fats and waxes.
4) The filtrate obtained from (1) is evaporating to approximately 1/10th its volume by heating in a water bath maintained at a temp less than 70^0C. It was acidified with 2M, H_2SO_4. The acidified filtrate is extracted using 75 CC (3 x 25 CC) chloroform in a separating funnel. The chloroform layer transferred to a dry pre-weighed beaker. The chloroform layer is evaporated to dryness on a water bath maintained at 45 ^{0}C$\pm$ 5^{o}C.
5) After evaporation of chloroform the beaker is allow to cool at room temp in dessicator. After cooling the weight of this beaker containing the residue. The residue obtained is reconstituted in chloroform to obtain a final conc. Of 10mg/CC. this extract filtered through filter paper No. 41. The filtrate obtained moderately polar extract, which consists of terpenoids.

6) The aqueous layer from (4) basified to pH = 10 with 2 M NaOH. It is further extracted with 60 CC (2 x 30 CC) chloroform method in volume ratio 3:1 followed by extraction with 40 CC (2 x 20 CC) chloroform in a separating funnel. The aqueous Basic layer transferred to a dry pre-weighed beaker. The aqueous Basic layer evaporated to dryness on a water bath maintained at 70^0C. After evaporation of the solvent the beaker allow to cool at room temp in a dessicator. After cooling, the weight of the beaker containing the residue will note down. The residue obtained in methanol to obtain a conc. of 10mg/cc. The extract filtered through Whatman filter paper No. 41. The methanol extract obtained the polar extract. The polar extract consists of quaternary alkaloids and N. oxides.
7) The organic layer (Chloroform and methanol) transferred to a dry and preweighed beaker on a water bath maintained at $45^0C \pm 5^0C$. After evaporation of solvent the beaker allowed to cool at room temperature in a dessicator. After cooling the weight of the beaker containing residue will noted down. The residue obtain reconstituted in chloroform to obtain final concentration of 10 mg/cc. Extract then filtered through Whatman filter paper No. 41.
8) Two grams of dried leaf powder extract with 1000cc MeOH (4 x 25 CC) in a dry stoppered concial flask. The filtrate obtain evaporate to dryness on water bath maintained at 70^0C. After evaporation, the beaker allowed to cool at a room temperature in a dessicator. After cooling, the weight of the beaker containing the residue will be noted .According to the condition of extract following parts or types of compound may be present Moderately polar extract (Terpenoids & Phenolic), Basic extract (Most Alkaloids,) Polar extract (Q. Alkaloids & N-oxides), Natural Extract (Fats and waxes), Fibers etc.

Plant Material

Healthy plant material like leaves, stem, root and matured fruits with seeds of *A. abutilon is* available from Shirur and Khed (Ambegaon), Baramati, Haveli and Maval Tahsil area from Pune District in State of Maharashtra, India. Identification of plants by referring the standard morphological characteristic features provides in the floras for the identification of the species.

Preliminary phytochemical analysis:

The preliminary phytochemical studies will be carried out by the standard methods described by Kokate *et al* with some small modification. The plant parts (Root, stem, leaf and matured fruits with seed) extract was assayed for the presence of alkaloids, Saponins, terpenoids, phenols.

Preparation of extracts:

Fresh leaves, stem, roots and fruits with seeds wash thoroughly under running tap water two to three times, removing all soil particles and shade dried under normal temperature. Dried leaves stem, roots and whole fruits with seeds were separately homogenized to a fine powder and stored in airtight bottles. 50gm of leaves, stem, roots and seeds powders were extracted with 1000 ml of solvent (Water, Ethanol, chloroform, Ether, Acetone and Methanol) for 42 hrs by using soxhlate apparatus. Use this extract for different phytochemical chemical tests.

Quantitative and Qualitative Phytochemical screening

Quantitative and qualitative phytochemical screening of plant according to standard procedures. Qualitative analysis of some phytochemicals such as:

Alkaloids

The presence of alkaloids shown by precipitation of salts and reaction with Mayer's reagent (potassium tetra-iodo mercurate solution). To 10 g of powdered sample, 50 ml of 10% sulphuric acid add. After 24 hrs at ambient temperature the mixture filter on filter paper and rinse with distilled water to obtain 50 ml of filtrate, 1 ml of which placed in a test tube and treated with five drops of Mayer's reagent.

Saponins

Crude extract mixed with 5ml of distilled water in a test tube and it shake vigorously. The formation of stable foam takes as an indication for the presence of saponins.

Terpenoids

The presences of terpenes demonstrate using concentrated sulphuric acid. An extract first prepare by maceration for 24 hrs of 1 g of powder sample and 20 ml of ether. The extract obtained used to detect carotenoids and coumarins in addition to sterols. Sterols and triterpenes were detected by adding 1 ml of chloroform to the residue left by evaporating 10 ml of the macerate. The solution obtained place in two test tubes, and 1 to 2 ml of concentrated sulphuric acid place at the bottom of one of them, the other acting as a control. The formation of a brownish-red or violet ring where the two phases met indicated their presence. Carotenoids, terpene pigments useful for photosynthesis, were detected using antimony trichloride (Bruneton, 1993). Two to three drops of a saturated solution of antimony trichloride ($SbCl_3$) in chloroform were added to take up the

residue left by evaporating 5 ml of ether extract to dryness. A blue coloration indicates their presence.

Phenols

Total phenolic content of the extract determine by Folin ciocalteu reagent method with some modification. Plants extract (1 ml) mixed with Ciocalteu reagent (0.1 ml, 1 N) and allowed to stand for 15 min, Then 5 ml of saturated Na_2CO_3 added. The mixture allows standing for 30 min at room temperature and the total phenols were determined spectrophotometrically at 760 nm. Gallic acid used as a standard. Total phenol value express in terms of Gallic acid equivalent (mg g-1 of extracted compound.)

General screening conduct to characterize chemical composition of *Abitulon indicum* root, leaf, stems and fruits with seed samples. Phenolic have attracted a great attention in relation to their potential for beneficial effects on health. Over the last few years, several experimental studies have revealed biological and pharmacological properties of phenolic compounds, especially their antimicrobial, antiviral, anti-inflammatory and cytotoxic activity.

Work plan

- **First six month**

1) Literature survey
2) Data Collection
3) Provision of chemicals for preparation of samples
4) Weighing of ingredients
5) Mixing and Milling of ingredients in their weight properties'
6) Preparation of sample
7) Pre-sintering and slow cooling
8) Milling of pre-sintered powder materials
9) Sintering and slow cooling
10) Milling and pellet formation of sintered powder materials
11) Final sintering of the samples
12) Milling, powder, Grinding

- **Second six month**

1) Characterization of powdered samples by TLC technique, Solubilisation in different solvents and analysis for the calculation of various parameters'.
2) Characterization of powdered samples by infrared absorption technique and analysis.
3) Characterization of powdered samples in animal house.
4) Course work and theory papers.

- **Third six month**

1) Detection of properties of samples by testing with mice.
2) Observations of animal effects.
3) Seeing effect of plant sample on other animals.
4) Analysis of Result and conclusions.

- **Fourth six months**

1) Writing of research papers and publication of research papers.
2) Thesis Writing.
3) Typing and binding of the research thesis.
4) Submission of thesis.